THE DIMORPHIC CLAWS OF THE HERMIT CRAB, *PAGURUS POLLICARIS*: PROPERTIES OF THE CLOSER MUSCLE

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ABSTRACT

The first pair of chelipeds of the flat clawed hermit crab (*Pagurus pollicaris*) are dimorphic. The crusher claw is always on the right side and is larger than the cutter claw on the left. The closer muscle in the crusher claw has a wet weight that is 3.4 times greater than that in the smaller cutter claw.

The closer muscle fiber types are different in the two claws. The crusher closer muscle has fibers with long (9–14 μm) sarcomeres, a high thin to thick filament ratio, and low myofibrillar ATPase activity; these fibers are presumably slow. The cutter closer, by contrast, has two types of fibers that are regionally distributed within the muscle. Fibers located on the dorsal and ventral margins of the muscle have long (8–13 μm) sarcomeres, a high thin to thick filament ratio, low myofibrillar ATPase activity, and are presumably slow fibers. In the central portion of the cutter closer muscle, however, there is a band of fibers with short sarcomeres and high myofibrillar ATPase activity. These features suggest that these fibers are fast. However, the high thin to thick filament ratio found in these "fast" muscle fibers would argue against this presumption. Finally, sarcomere length measurements taken from closer muscle fibers in other thoracic appendages revealed a bimodal distribution of fiber types similar to that observed in the cutter claw.

INTRODUCTION

Although bilateral symmetry is one feature that has been conserved during the evolution of higher animals, some species exhibit bilateral asymmetry. Perhaps the most notable examples can be found in certain crustaceans, where the first pair of chelipeds have become enlarged to form claws. Claw asymmetry can be found in crustaceans such as sexually mature male fiddler crabs (*Uca*), pistol or snapping shrimp (*Alpheus*), the American lobster (*Homarus*), and certain hermit crabs (*Pagurus*). Recently, many investigators have used these crustaceans to study the development of asymmetry in otherwise bilaterally symmetrical animals.

In lobsters and snapping shrimp collected from the wild, as many animals have the major claw on the left side as on the right (Przibram, 1931). Moreover, in these animals the dimorphic claws have different behavioral functions and associated contrasting contractile properties in the claw musculature. In lobsters, for example, the larger "crusher" claw is used for crushing mollusc shells (Herrick, 1895) and has a closer muscle composed of slow tonic fibers capable of producing large amounts of tension (Govind and Lang, 1974; Lang *et al.*, 1977a). The smaller "cutter" claw, by contrast, can be closed rapidly and has a closer muscle with a large proportion of fast, phasic fibers.

In snapping shrimp, the smaller "pincer" claw is used for manipulation of small objects, while the larger "snapper" claw is used during territorial encounters with other shrimp (Schein, 1975). The pincer claw closer muscle is composed of intermediate and fast muscle fibers (Stephens and Mellon, 1979), which are regionally distributed within the muscle (O'Connor *et al.,* 1982); the fast fibers form a central band along the longitudinal axis of the muscle. The snapper claw closer muscle, however, is composed of a uniform population of slow, tonic fibers. An interesting feature of adult snapping shrimp is an ability to reverse claw configuration (Wilson, 1903; Mellon and Stephens, 1978). Removal or denervation of the snapper claw causes the contralateral pincer claw to enlarge and transform into a snapper claw. The process involves modifications of the external skeletal arrangements and an increase in the mass of the claw musculature. In addition, in Floridian species, there is a change in the properties of the closer muscle fibers from fast and intermediate to slow (Mellon and Stephens, 1980), and there is a parallel increase in the degree of facilitation exhibited by the excitatory motor neurons (Stephens and Mellon, 1979). These results have provoked the suggestion that neurotrophic influences underlie claw transformation in snapping shrimp.

In the dimorphic claws of hermit crabs (*Pagurus pollicaris*) the larger claw is always found on the right side. This may be explained by the right-handed spiral of the gastropod shells in which they live. Such a shell orientation would exert less spatial constraint on the development of the claw on the right side. In the present investigation we have examined the fiber composition of the closer muscles in the dimorphic claws using morphological, histochemical, and biochemical properties and find them to be different.

MATERIALS AND METHODS

Flat clawed hermit crabs (*Pagurus pollicaris*) were obtained commercially from Woods Hole, Massachusetts, or from Panacea, Florida. In the laboratory, the animals were kept in aquaria filled with artificial sea water at a temperature of 15–20°C. The animals were fed frozen brine shrimp or Tetramin fish food, and were usually used for experimentation within two weeks of arrival at the laboratory. The study involved analysis of muscle fibers types by examining myofibrillar adenosinetriphosphatase (ATPase) activity from frozen sections (Ogonowski and Lang, 1979; O'Connor *et al.,* 1982), and by measuring sarcomere length from teased myofibrils (Atwood, 1973, 1976; Josephson, 1975).

The histochemical properties of the closer muscles in pairs of claws were examined using standard techniques (Ogonowski and Lang, 1979; O'Connor *et al.,* 1982). Since the cuticle of the propus is thick, it was necessary to remove the shell surrounding the closer muscle prior to freezing in liquid nitrogen and sectioning.

Sarcomere length measurements were made for the closer muscles in all of the thoracic appendages, using methods described elsewhere (Lang *et al.,* 1977a; O'Connor *et al.,* 1982). Briefly, the animal was removed from its shell and made to autotomize a limb by applying pressure at the base. The limb was soaked in a saline solution (Chapple, 1977) in which the calcium ions had been replaced with magnesium, to prevent measurement errors due to muscle contraction. The dactyl was fixed in the open position and the limb was bathed in alcoholic Bouin's solution for two days. The limb was then stored in 80% ethanol. Individual fibers were removed from the closer muscle and were teased apart in 80% ethanol on a microscope slide. The myofibrils were examined under a compound microscope fitted with Nomarski optics. A calibrated ocular micrometer was used to measure the length of five consecutive

sarcomeres; at least three measurements were made for each muscle fiber and the average length of a sarcomere was calculated for each muscle fiber.

Electron microscopy was performed on the closer muscle of the cutter and crusher claws. The cuticle on the ventral surface of the propus together with the underlying opener muscle were removed from an autotomized claw. Small holes were drilled through the cuticle of the propus and the dactyl was fixed in the open position. The claw was soaked in the calcium-free saline and then fixed for 60 min in a solution containing glutaraldehyde (2.5%), formaldehyde (0.2%), sucrose (300 mM), sodium chloride (60 mM), calcium chloride (2 mM), and sodium cacodylate (150 mM, pH 7.2). Individual muscle fibers were removed from the closer muscle and fixed in the same solution for an additional 30–60 min. The muscle fibers were washed in the same solution (without fixatives), post-fixed in 2% osmium tetroxide, and embedded in epon for thin sectioning on an ultramicrotome.

RESULTS

The crusher claw on the right side is larger than the contralateral cutter claw (Fig. 1A, B) and has a closer muscle of greater mass. In seven pairs of claws the wet weight of the closer muscle in the crusher claw was 3.4 (S.D. ± 0.3) times larger than in the cutter claw (cutter closer muscle weight 27.5–141 mg).

Measurement of sarcomere lengths of fibers in the crusher closer muscle gave values between 9 and 14 μm (Fig. 1F). The homogeneous population of long sarcomeres indicates that the closer muscle in the larger crusher claw is composed of slow fibers (Atwood, 1973, 1976; Josephson, 1975). In the cutter closer muscle, by contrast, sarcomere length analysis revealed a bimodal distribution of fiber types: short sarcomere (2–4 μm), presumably fast fibers, and long sarcomere (8–13 μm), presumably slow fibers. Moreover, data from fibers selectively removed from different regions of the cutter closer muscle showed that fast fibers are located primarily in the central portion of the muscle (Fig. 1E), although some are found ventrally (Fig. 1C). Slow fibers, however, are situated on the dorsal and ventral margins of the muscle (Fig. 1C, D).

Table I gives closer muscle sarcomere length data for the five pairs of thoracic appendages from one animal; for each muscle, fibers were removed from dorsal, ventral, and central regions. It is interesting that in the walking (2 and 3) and the vestigial (4 and 5) limbs there is a bimodal distribution of fiber types, which is similar to that observed in the cutter claw closer muscle.

Electron microscopy of closer muscle fibers from the cutter and crusher claws confirmed the sarcomere length data obtained from light microscopy. In addition, cross sections permitted analysis of the relationship between the thick and thin filaments. In long sarcomere fibers removed from cutter and crusher claw closer muscles each thick filament is associated with 10–14 thin filaments (Fig. 2A–D). This arrangement is typical of slow or tonic muscle (Atwood, 1973). In cutter closer muscle fibers with short sarcomeres (Fig. 2E), presumably fast fibers, each thick filament is associated with 7–10 thin filaments (Fig. 2F). This arrangement of thick and thin filaments is not typical of fast phasic fibers (Atwood, 1973).

Figure 3 shows frozen transverse sections of a closer muscle from a cutter claw stained for myofibrillar ATPase activity. Fibers located in the central region of the cutter closer muscle were dark staining, while those on the dorsal margin were generally light staining. On the ventral margin of the muscle some fibers stained darkly while others were light staining for myofibrillar ATPase activity. It has been shown in other crustacean muscles that fibers with high myofibrillar ATPase activity (*i.e.*, dark staining) are phasic or fast, while light staining fibers are tonic or slow (Ogonowski and Lang,
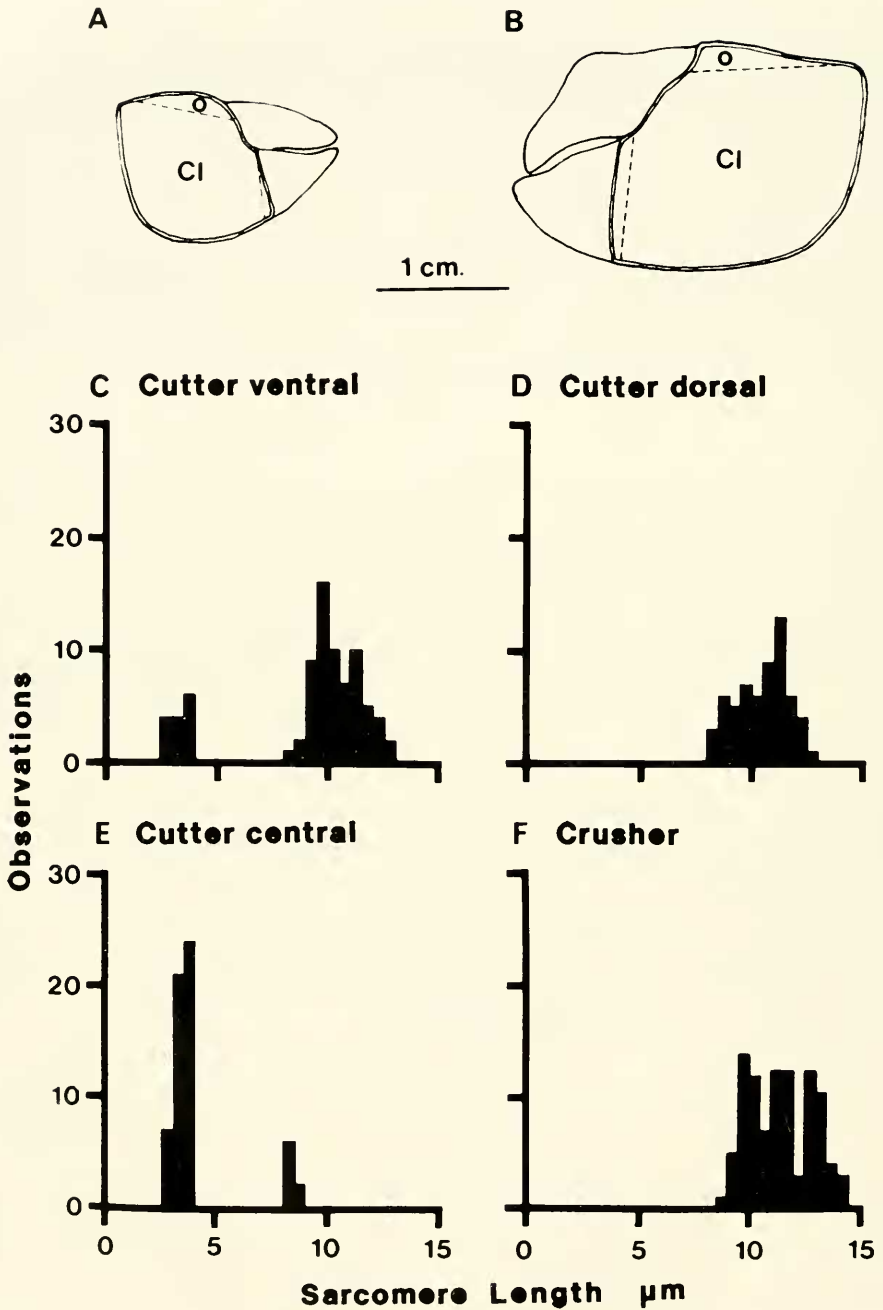
FIGURE 1.   Sarcomere length measurements for closer muscle fibers in the crusher and cutter claws of a hermit crab. A, B: Diagrams of the cutter and crusher claws, respectively, showing their relative sizes and the locations of the closer (Cl) and opener (O) muscles. C–F: The sarcomere lengths of closer muscle fibers removed from different regions of the clutter claw (judged by eye) and from the crusher claw. Measurements were made from 250 fibers in the cutter and 100 fibers in the crusher claw.

TABLE I

The sarcomere lengths of closer muscle fibers from the five thoracic limbs of a hermit crab (Pagurus pollicaris)[1]

Thoracic limb	Right	Left
1	11.6 (1.7)	10.3 (1.3)
		3.6 (0.5)
2	10.1 (1.3)	10.0 (0.5)
3	3.8 (0.4)	4.4 (0.3)
	9.5 (0.4)	9.4 (0.3)
4	3.8 (0.1)	4.2 (0.3)
	9.6 (0.2)	9.4 (1.1)
5	4.2 (0.3)	4.3 (0.2)
	8.6 (0.4)	8.4 (0.4)
	4.2 (0.2)	4.3 (0.2)

[1] The sarcomere lengths of 25 fibers in each muscle were measured and the values represent mean sarcomere lengths (± standard deviation). Note that the first thoracic limb on the right side is a crusher and on the left is a cutter.

1979; Silverman and Charlton, 1980; O'Connor *et al.,* 1982). Our results, coupled with the sarcomere length measurements (Figs. 1, 2), indicate that the crusher closer muscle is composed of slow tonic fibers. The cutter closer has slow fibers on the dorsal and ventral margins, and fast fibers located ventrally and centrally, forming a band along the longitudinal axis of the muscle. Our data, therefore, demonstrate a regional distribution of different fiber types in the cutter claw closer muscle of the hermit crab. However, the distribution is not as discrete as that found in the closer muscles of the smaller claws of snapping shrimp (O'Connor *et al.,* 1982) and lobster (Ogonowski *et al.,* 1980), where the fast fibers form discrete bands in the muscle.

DISCUSSION

The present study of the dimorphic claws of the hermit crab (*Pagurus pollicaris*) shows that the closer muscles have different morphological and histochemical characteristics, from which certain contractile properties can be concluded (Atwood, 1973). The crusher closer muscle is composed of an homogeneous population of slowly contracting tonic fibers, while the cutter closer has two types of fibers, fast and slow (Figs. 1–3). The different types of fibers are regionally distributed within the cutter closer so that the fast fibers form a band along the longitudinal axis of the claw, in the central and ventral portions of the muscle (Fig. 3). However, it is interesting that the presumptive fast cutter closer muscle fibers, although they have high myofibrillar ATPase activity and short sarcomeres, also have a thick to thin filament ratio that is not typical of fast fibers. To our knowledge this is the first report of a fast adult crustacean that contrasts with the "classical" arrangement of one thick filament surrounded by six thin filaments fibers (Atwood, 1973).

It is interesting that a similar regional distribution of fiber types has been found in the closer muscle of the smaller chelipeds of other crustaceans with dimorphic claws, most notably snapping shrimp (O'Connor *et al.,* 1982) and lobsters (Ogonowski
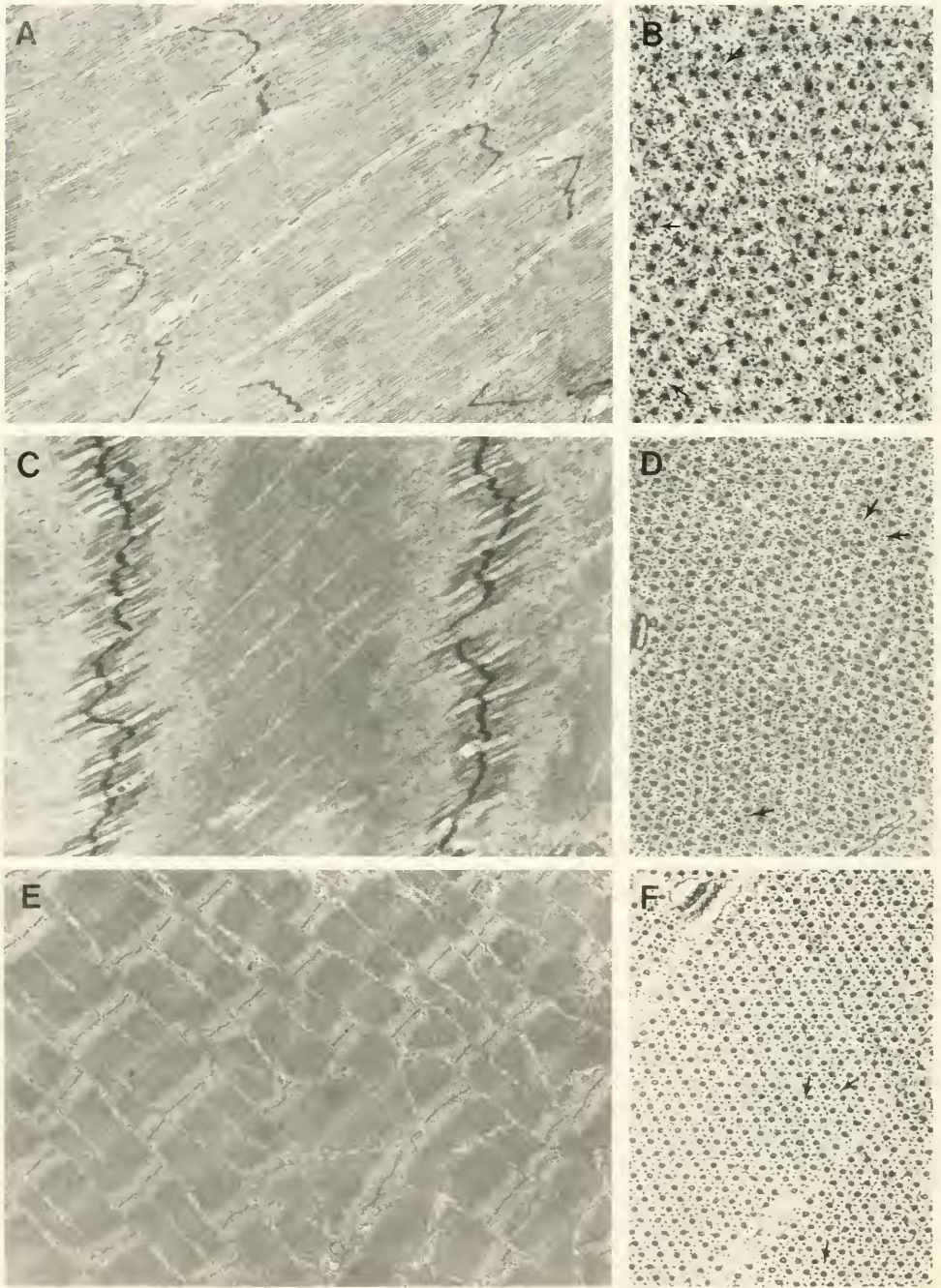
FIGURE 2.   Electron micrographs of longitudinal (A, C, E) and cross sections (B, D, F) of claw closer muscle fibers. Sections were taken from a crusher closer muscle (A, B), and a long sarcomere (C, D) and a short sarcomere fiber (E, F) taken from a cutter claw. Orbits where the number of thin filaments surrounding a thick filament can be clearly seen are indicated by arrows. Calibration: 3 μm (A, C, E,) and 0.5 μm (B, D, F,).
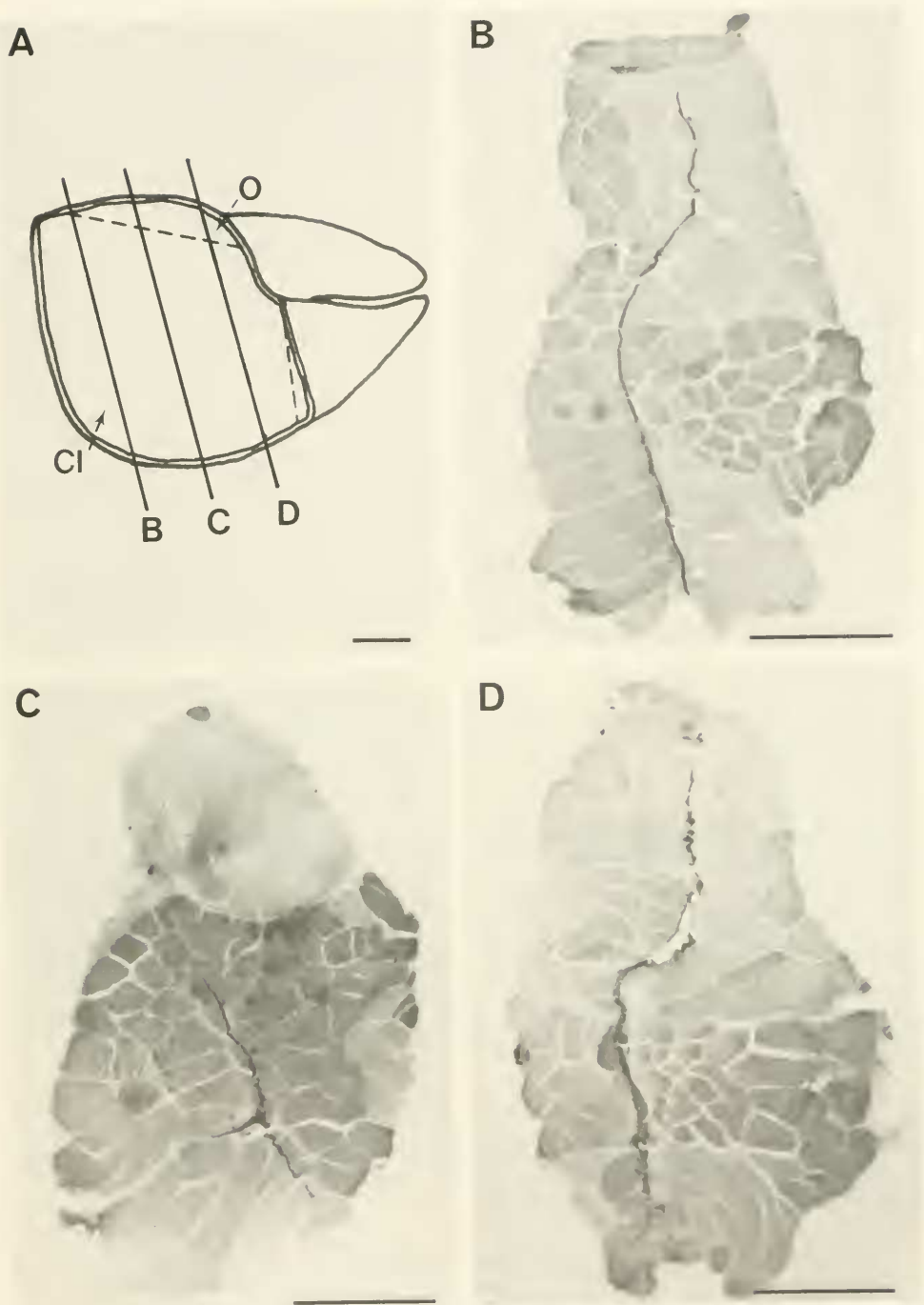
FIGURE 3. Myofibrillar ATPase activity of the cutter closer muscle. The diagram of the cutter claw shows the relative positions of the opener (O) and closer (Cl) muscles in the claw, and the levels at which the sections (B–D) were taken. Note that in all plates the dorsal surface is uppermost and that the shell was removed prior to sectioning of the muscle. Calibration: 100 μm.

et al., 1980). Moreover, sarcomere length measurements made from thoracic limb closer muscles have shown a bimodal distribution of fiber types in lobster (Govind *et al.,* 1981) and in hermit crabs (Table I). These observations suggest that the bimodal distribution of fast and slow fibers may be a common (or even primitive) feature of the crustacean closer muscle. According to this hypothesis, therefore, the enlargement of the right claw of the hermit crab to become the crusher not only involves an increase in the mass of the closer muscle, but also results in the loss of fast fibers. Work on the development of the asymmetric claws of lobsters supports this hypothesis. In larval and early juvenile lobsters, the claws are symmetric and the closer muscle in each claw has fast and slow fibers (Lang *et al.,* 1977b). According to the above hypothesis, therefore, the bimodal distribution of fiber types suggests that during early stages of lobster development the closer muscle fiber types are primitive in both claws. During normal growth the claws become dimorphic and the closer muscles become asymmetric. In the cutter claw closer muscle there is an increase in the number of fast fibers. In the crusher claw closer muscle, by contrast, there is an increase in the number of slow fibers and a decrease (and ultimately a disappearance) of the fast fibers (Ogonowski *et al.,* 1980). Therefore, during normal lobster claw development the specialization of the crusher claw involves skeletal modifications and the loss of fast closer muscle fibers.

Asymmetry has been reported in other regions of the hermit crab besides the claws. There are no abdominal pleopods on the right side, and those that remain on the left have become simplified (Bent and Chapple, 1977a); there has been a concomitant reduction in the number of motor neurons on the right side (Bent and Chapple, 1977b). Similarly, the mass of the deep flexor muscles on the right side is greater than on the left (Marrelli, 1975). It may be argued that reduction of the left side is due to spatial constraints exerted by the right-handed whorls of the gastropod shells in which the crabs live. Similarly, in the case of the dimorphic claws, it seems reasonable that the greater space on the right side favors the location of the larger crusher on this side. However, Chapple (1979) has described dimorphism in the claws as early as the megalops stage, prior to the hermit crab's entry into gastropod shells. In lobster and snapping shrimp claw dimorphism is associated with asymmetries in claw function (Herrick, 1896; Przibram, 1931) and neuromuscular properties (Govind and Lang, 1974; Lang *et al.,* 1977a; Stephens and Mellon, 1979; O'Connor *et al.,* 1982). The present paper reports similar asymmetries in properties of the closer muscles in the dimorphic claws of hermit crabs. Moreover, cursory observations have revealed that there is an asymmetry in the roles of the dimorphic claws during feeding on Slipper shells (*Crepidula*). The major claw is used for holding the shell while the minor claw picks at and transfers the flesh to the mouth.

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